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Occupational exposure in ANCA-positive patients: A case-control study

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Background. Antineutrophil cytoplasmic autoantibodies (ANCA) are valuable biomarkers for the diagnosis and follow-up of small vessel vasculitis. The role of ANCA has not yet been fully established, but genetic, infectious, and/or environmental factors may increase susceptibility to these diseases. We performed an epidemiologic study to investigate whether the presence of ANCA was associated with silica or any other form of occupational exposure, regardless of the underlying disease.

Methods. All consecutive ANCA-positive patients recorded at the institution's Laboratory of Immunology between 1990 and 2000 were included. Patients hospitalized in a unit of internal medicine matched for age and gender were selected as controls (two controls/case). Qualitative and semiquantitative professional exposure and smoking habits were analyzed by five experts blind to the diagnosis.

Results. Univariate analysis showed that patients who reported dust exposure had a 2.6 greater risk of being ANCA-positive ($P = 0.007$) (odds ratio 2.6; 95% CI 1.3 to 5.3) and individuals with professional exposure to silica had a 3.4 higher risk of being ANCA-positive ($P = 0.03$) (odds ratio 3.4; 95% CI 1.1 to 9.9). None of the other environmental factors or smoking habits were different between ANCA-positive patients and controls. There was no difference in silica exposure between patients with cytoplasmic ANCA (c-ANCA), perinuclear ANCA (p-ANCA), or atypical ANCA.

Semiquantitative analysis showed a dose effect of silica exposure with a nearly sevenfold greater risk of being ANCA-positive compared to controls ($P = 0.02$) (odds ratio 6.9; 95% CI 1.3 to 35.1).

Conclusion. These results support the hypothesis that the presence of ANCA in plasma might at least partially be related to occupational exposure.

Key words: antineutrophil cytoplasmic antibodies, occupational exposure, silica, case-control study.

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Antineutrophil cytoplasmic autoantibodies (ANCA) are frequently associated with small vessel vasculitis such as Wegener granulomatosis, Churg and Strauss syndrome, microscopic polyangiitis, pauci-immune glomerulonephritis, and Goodpasture syndrome [1]. ANCA serve as biomarkers for the diagnosis and follow-up of these diseases but they are also detected in the serum of patients suffering from other diseases such as autoimmune rheumatism [2], chronic inflammatory intestinal disease [3], Sjogren syndrome [4], autoimmune hepatitis [5], and lupus [6]. Antithyroid drug-induced ANCA-associated vasculitis has also been reported [7].

ANCA are biomarkers which are detected on indirect immunofluorescence assay and three different types of fluorescence have been identified [i.e., cytoplasmic ANCA (c-ANCA), perinuclear ANCA (p-ANCA), and atypical ANCA] [8, 9]. The cytoplasmic antigen targets of each of these antibodies are different. c-ANCA are mostly associated with antiproteinase 3 (PR3) antibodies whereas p-ANCA are associated with antimyeloperoxidase (MPO) antibodies. Wegener granulomatosis is predominantly associated with c-ANCA (75%), while microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune glomerulonephritis, and Goodpasture syndrome are predominantly associated with p-ANCA (50%, 60%, 70%, and 25%, respectively) [9].The antigen specificity of atypical ANCA is less clearly defined [9].

The precise role of ANCA in ANCA-associated diseases has not yet been clearly established [10, 11]. Some studies using animal models have suggested that the anti-MPO antibody would alone be capable of creating necrotizing glomerulonephritis [12]. MPO knockout mice immunized with murine MPO developed an anti-MPO antibody response. Small vessel vasculitis and necrotizing and crescentic glomerulonephritis developed when splenocytes from these animals were transplanted to RAG2 mice [11].

Certain factors, particularly the genetic, infectious and environmental factors, may influence the occurrence of

such diseases. Indeed an association between severe and moderate protease inhibitor-deficient phenotypes and ANCA-positive vasculitis has been described [13] and cotrimoxazole has been reported to prevent relapses of vasculitis [14].

Environmental factors, particularly silica exposure, have been reported to be associated with certain forms of ANCA-associated vasculitis [15, 16] and with several autoimmune diseases such as scleroderma [17], rhumatoid arthritis [18, 19], dermatomyositis [8], and lupus [6].

The aim of this study was to determine whether the presence of ANCA in plasma is associated with silica exposure regardless of the underlying disease. As this study was performed through a panel of experts, we analyzed any occupational exposure to toxic agents in these subjects.

METHODS

Detection of ANCA

All ANCA were detected in serum by indirect immunofluorescence in the same laboratory according to the standard procedure established at the first ANCA workshop [20]. Positive indirect immunofluorescence was classified as c-ANCA, p-ANCA, or atypical ANCA (Inova, San Diego, CA, USA). Sera positive for p-ANCA or atypical ANCA were also tested for antinuclear activity on Hep 2 cells. Antigen target specificity of ANCA for PR3 or MPO was systematically investigated using a commercially available enzyme-linked immunoabsorbent assay (ELISA) (Pharmacia, Freiburg, Germany) since 1996.

Selection of ANCA patients and controls

All patients admitted to the Tours University Hospital between 1990 and 2000 with the first known serum ANCA levels at a dilution rate of at least 1/20 were included, regardless of specificity and/or the underlying disease. A total of 97 patients were ANCA-positive during the study period. Thirty-seven patients were excluded from the study for loss to follow-up ($N = 13$) (13.4%), refusal to participate ($N = 6$) (6.2%), death ($N = 18$) (18.6%), leaving a total of 60 patients (Fig. 1).

The diseases which were diagnosed in the 60 ANCA-positive patients included Wegener granulomatosis according to the 1990 American College of Rheumatology ($N = 20$) [21], microscopic polyangiitis ($N = 8$), pauci-immune glomerulonephritis ($N = 10$), and uveitis ($N = 6$), and also Horton disease ($N = 2$) and stroke ($N = 4$) (Table 1).

Control subjects were selected from patients admitted to the Department of Internal Medicine in the same hospital between 2000 and 2001 and matched with ANCA-positive patients on the basis of age (within 5 years) and gender. Two control subjects were selected for each ANCA-positive patient. All control subjects were tested for ANCA in the same laboratory and all were confirmed to be negative. Controls were in-patients with diabetes ($N = 76$), cardiovascular diseases ($N = 25$), or miscellaneous other diagnosis ($N = 19$). Patients treated with neomercazole or suffering from infectious diseases (particularly endocarditis), antiphospholipid antibodies, or malignancies were excluded from the study to avoid patients with false positive titers with ANCA.

Table 1. Diseases in 60 antineutrophil cytoplasmic autoantibodies (ANCA)-positive patients

Disease	Cytoplasmic ANCA $N = 20$	Perinuclear ANCA $N = 29$	Atypical ANCA $N = 11$	Total $N = 60$
Wegener disease	13	7	0	20
Pauci-immune glomerulonephritis	0	7	3	10
Microscopic polyangiitis	1	6	1	8
Uveitis	3	1	2	6
Churg and Strauss syndrome	0	3	1	4
Stroke	2	1	1	4
Horton disease	0	2	0	2
Periarteritis nodosa	1	1	0	2
Circulating anticoagulant	0	1	0	1
Autoimmune hepatitis	0	0	1	1
Rheumatoid arthritis	0	0	1	1
Hemorrhagic rectocolitis	0	0	1	1

Exposure

Occupational exposure was evaluated by a standard interviewer-administered questionnaire. The structured questionnaire consisted of questions on life style such as smoking and on work. For each job lasting for periods longer than 6 months, job status, company name, years worked, detailed description of tasks performed and working environment, including information regarding dust and exposure (silica dust, silicon, organic solvent, and any other known toxic agent), were reported. A panel of five experts blind to the diagnosis (three occupational physicians, one epidemiologist, and one industrial hygienist) studied the responses questionnaires for each patient together. Analysis of occupational exposure was both qualitative and semiquantitative. An exposure score was calculated for each person's employment periods during which exposure to each toxic agent occurred. This exposure score took into account the probability of exposure (probability score of 0, nonexposure; 0.25, possible exposure; 0.75, probable exposure; and 1, certain exposure), intensity of exposure (intensity score from 0 for nonexposure to 4 for highest level of exposure), frequency of exposure (with a frequency score based on length of time worked daily <10%, 0.05; 10% to 50%, 0.30; and >50%,

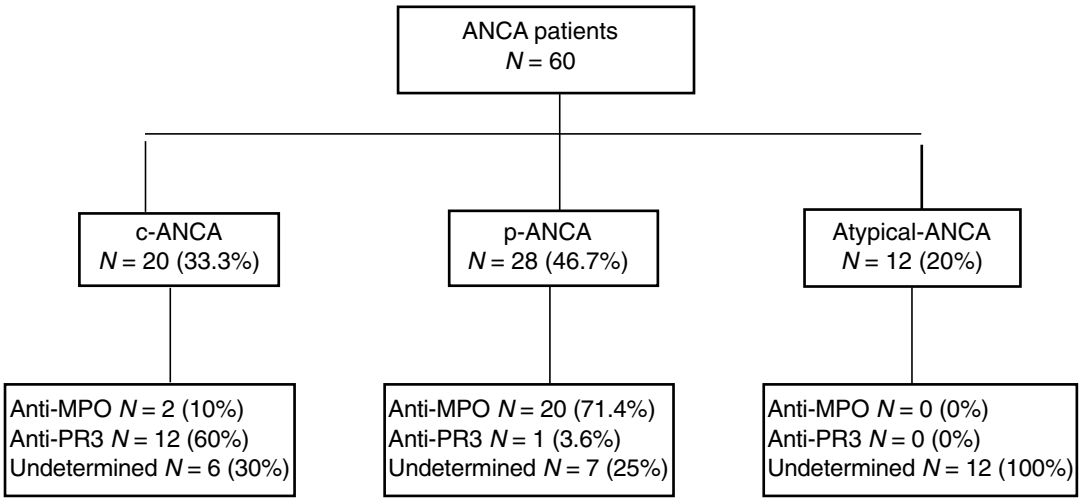

Fig. 1. Patients enrolled in the study, with type and specificity of antineutrophil cytoplasic antibodies (ANCA). Abbreviations are: anti-MPO, antimyeloperoxidase antibodies; anti-PR3, antiproteinase 3 antibodies; c-ANCA, cytoplasmic ANCA; p-ANCA, perinuclear ANCA.

0.75) and duration of exposure (number of years worked). Finally, the exposure score for each employment period was expressed as the product of probability × intensity × frequency × duration. The final cumulative exposure score for each subject was expressed as the sum of [17, 22] employment exposure scores, taking into account all periods of employment. Four increasing degrees of occupational exposure score were defined: nil, low (score <2), moderate (2 to 4), and high (score >4). Exposure periods that were considered for statistical analysis of cases and controls were the same and preceded the diagnosis of diseases.

Statistical analysis

Conditional logistic analysis was performed to estimate the relation between the different variables by univaraite and multivariate analysis. Maximum likelihood were estimated using odds ratio (OR) and confidence intervals (95% CI).The Armitage trend test was used to evaluate whether there was a dose and effect relationship between the cumulative silica exposure and the presence of the biomarker ANCA [23]. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

A total of 180 patients responded, of whom 60 were ANCA-positive (cases) and 120 were control subjects (controls). As expected, age and gender were comparable between ANCA-positive patients and control subjects (mean ± SD 61.2 ± 12.5 years vs. 61.8 ± 12.6 years and 30 males/30 females vs. 60 males/60 females, respectively). All cases and controls lived within 200 km of the hospital.

Occupational exposure

Self reported. ANCA-positive patients reported significantly more frequent exposure to dusty environments during their working lives than control subjects (OR = 2.6; 95% CI 1.3 to 5.3) ($P = 0.007$). There was no difference between the two groups for recent or previous smoking history (Table 2).

Qualitative analysis. Qualitative analysis of work exposure by the panel of experts showed that ANCA-positive patients were significantly more often exposed to silica than the control subjects (OR = 3.4; 95% CI 1.1 to 9.9) ($P = 0.03$). Twenty-five percent of the c-ANCA-positive patients ($N = 5$), 21% of the p-ANCA-positive patients ($N = 6$) and 17% of the atypical ANCA-positive patients were exposed to silica ($P = 0.34$). There was no significant difference between ANCA-positive patients and control subjects in the exposure to any other toxins evaluated by the experts, in particular to white spirit, silicone, benzene, paint, or lead (Table 3). There was also no significant difference between groups on pooling chlorate solvents (trichlorethylene, trichlorethan, and tetrachlorethylene) (OR 1.5; 95% CI 0.7 to 3.1) ($P = 0.30$), aromatic solvents (benzene, toluene, and xylene) (OR 1.4; 95% CI 0.6 to 3.2) ($P = 0.46$), and all solvents together (OR 1.6; 95% CI 0.8 to 3.2) ($P = 0.22$).

Quantitative analysis. Quantitative analysis showed a dose-effect relationship between silica exposure and the ANCA biomarker. When four groups of silica exposure of increasing intensity were defined the OR increased with intensity of silica exposure (trend test $P < 0.01$) (Table 4). The OR was statistically significant for high silica exposure (OR = 6.9; 95% CI 1.3 to 35.1) ($P < 0.01$).

Delay and type of silica exposure. In exposed patients to silica, duration of silica exposure was 16.3 years (± 12.5) for ANCA-positive patients ($N = 13$) compared

Table 2. Qualitative analysis of self-reported smoking and self-reported occupational dust exposure in antineutrophil cytoplasmic autoantibodies (ANCA)-positive patients and matched controls

	ANCA patients $N = 60$	Controls $N = 120$	Odds ratio (95% CI)	*P* value
Smoking				
Nonsmoker (%)	34 (56.6)	68 (56.7)	1	
Previous (%)	7 (11.7)	23 (19.2)	0.6 (0.2–1.6)	0.4
Current (%)	19 (31.7)	29 (24.1)	1.3 (0.6–2.7)	
Occupational dust exposure (%)	29 (48.3)	34 (28.3)	2.6 (1.3–5.3)	0.007

Table 3. Risk associated with occupational exposure in 60 antineutrophil cytoplasmic autoantibodies (ANCA)-positive patients and 120 matched controls evaluated blind by five experts

Toxics	ANCA patients number (%)	Controls Number (%)	Odds ratio (95% CI)	*P* value
Silica	13 (21.7)	13 (10.8)	3.4 (1.1–9.9)	0.03
Silicone	10 (16.7)	19 (15.8)	1.1 (0.4–2.9)	0.87
Toluene	4 (6.7)	11 (9.1)	0.6 (0.2–2.4)	0.52
Trichlorethylene	11 (18.3)	21 (17.5)	1.1 (0.5–2.4)	0.89
White spirit	14 (23.3)	18 (15)	1.9 (0.8–4.3)	0.15
Benzene	7 (11.7)	12 (10)	1.2 (0.4–3.2)	0.73
Tetrachlorethylene	5 (8.3)	5 (4.1)	2.0 (0.6–6.9)	0.27
Trichlorethane	4 (6.7)	1 (0.8)	8.0 (0.9–71.6)	0.06
N hexane	2 (3.3)	5 (4.1)	0.2 (0.1–4.5)	0.77
Epoxy resin	7 (11.7)	9 (7.5)	1.9 (0.6–6.5)	0.29
Pesticide/herbicide	10 (16.7)	16 (13.3)	1.33 (0.553.07)	0.54
Solder	8 (13.3)	13 (10.8)	1.3 (0.5–3.7)	0.60
Asbestos	9 (15)	15 (12.5)	1.3 (0.5–3.2)	0.63
Petrol	11 (18.3)	18 (15)	1.3 (0.5–3.1)	0.55
Oil	11 (18.3)	21 (17.5)	1.3 (0.5–3.1)	0.88
Xylene	6 (10)	11 (9.2)	1.1 (0.5–2.5)	0.84
Formalin	6 (10)	6 (5)	2.2 (0.6–7.2)	0.21
Glue	4 (6.7)	13 (10.3)	0.6 (0.2–1.9)	0.38
Paint	2 (3.3)	6 (5)	0.6 (10^{-4}–3.5)	0.57
Glycol ether	6 (10)	9 (7.5)	1.4 (0.5–4.0)	0.57
Lead	3 (5)	9 (7.5)	0.6 (0.2–2.5)	0.50
Vegetable dust	6 (10)	18 (15)	0.6 (0.2–1.7)	0.36
Aromatic amine	4 (6.7)	2 (1.7)	4.0 (0.7–21.8)	0.11
Carbon oxide	1 (1.7)	2 (1.7)	1.0 (10^{-7}–11.0)	1

Maximum likelihood estimate of the odds ratio (OR) and confidence intervals (95% CI).

Table 4. Risk associated with final high, moderate, low or nil cumulative silica exposure score in patients with antineutrophil cytoplasmic antibodies (ANCA) and matched controls

Score	ANCA patients number (%)	Controls number (%)	Odds ratio (95% CI)	*P* value
Nil	47 (78.3)	107 (89.1)	1	
Low	2 (3.3)	6 (5)	0.8 (0.15–3.9)	0.74
Moderate	5 (8.3)	5 (4.2)	2.3 (0.63–8.24)	0.20
High	6 (10)	2 (1.6)	6.9 (1.33–35.09)	0.002

Maximum likelihood estimated using odds ratio (OR) and confidence intervals (95% CI).

Trend test $P = 0.009$.

to 19.4 years (±15.7) in control subjects ($N = 13$). The jobs associated with silica exposure in ANCA-positive patients were bricklaying ($N = 7$), foundry work ($N = 3$), laying tiles ($N = 2$), and there was one coal merchant, whereas control subjects included bricklayers ($N = 5$, dental prosthetist ($N = 1$), brick makers ($N = 5$) and those who lay tiles ($N = 2$). The interval between the beginning of silica exposure and discovery of ANCA was 36.3 years ±13.7 years (mean ± SD). The interval between the end of silica exposure and discovery of ANCA was 19.5 ± 19.5 years (mean ± SD).

DISCUSSION

We sought to determine whether the presence of ANCA is associated with work exposure, regardless of the underlying disease. The incidence of ANCA in the general population has not yet been fully established and might be influenced by geographic factors [24]. A case-control study was therefore designed in view of the low prevalence of this biomarker [25], which is an epidemiologic tool to reveal disease etiology [26] in the general population.

Several studies have been conducted to evaluate the risk of occupational exposure and vasculitis, whether associated with ANCA or not. An association between rapidly progressive ANCA-positive glomerulonephritis

and exposure to silica dust was studied in 16 patients and compared to 32 controls [27]. The authors reported a 14-fold greater risk of glomerulonephritis in silica-exposed patients. Of the seven patients who reported a history of work exposure to silica dust and who suffered from acute nephritis, six were p-ANCA-positive and one had no ANCA. In a case-control study, Nuyts et al [28] evaluated the role of occupational exposure in the occurrence of Wegener granulomatosis with renal involvement. Sixteen patients (13 c-ANCA-positive and three undetermined) were compared with 32 age- and gender-matched controls. Occupational exposure was scored by an industrial hygienist. Inhalation of silicon-containing compounds such as silica was associated with a nearly sevenfold risk of Wegener glomerulomatosis [28], whereas exposure to lead or cadmium was not associated with this disease. As in the present study the authors described considerable variability between the beginning of exposure to silica and the occurrence of disease.

Occupational exposure was recently compared between 101 patients suffering from Wegener glomerulomatosis and 123 controls, including 54 healthy controls, 24 patients with sarcoidosis or idiopathic pulmonary fibrosis, and 45 patients with inflammatory rheumatism. Patients with Wegener glomerulomatosis were significantly more often exposed to grain dust or fumes than controls ($P < 0.001$) but the presence of ANCA was not reported [15]. Finally, professional exposure was compared between 65 patients with ANCA vasculitis (34 with c-ANCA and 31 with p-ANCA) and 65 healthy controls in a multicenter study. The OR of silica dust exposure was 4.4 times greater for patients with ANCA vasculitis compared to control subjects [16]. However, in this study the testing for ANCA and MPO and PR3 specificity were performed in several centers, whereas in our study all analyses were performed at the same institution. Indeed standardization of ANCA testing between different laboratories is necessary to avoid misinterpretation of results [29]. Interestingly, as in our study, there was no association between smoking habits and ANCA-associated disease. Finally, the impact of environmental factors was reported in 75 patients suffering from primary systemic vasculitis (58 with ANCA and 17 without ANCA) in a case-control study [30]. A significant association between farming and vasculitis was identified.

The present study showed a significant association between silica and the presence of ANCA. This is the first study to our knowledge that demonstrates an association between a biomarker of autoimmune disease and silica exposure. There was no difference between specificity of ANCA and silica exposure. The way silica exposure might influence the occurrence of ANCA has not yet been clearly established. ANCA-related vasculitis might be related to a disease process in which accelerated apoptosis of polynuclear neutrophils and macrophages may act as a trigger [31]. Accelerated apoptosis has been induced through intratracheal instillation of silica in Wistar rats in a dose-dependent manner [32]. Moreover, in vitro studies have shown that silica may induce apoptosis in human peripheral blood lymphocytes, with an increase in Fas-mediated cell death after stimulation with silicate [33].

The predominant role of silica in the association with ANCA is confirmed by the fact that none of the other components studied was significantly different between patients and controls. Despite a very wide range of products examined blind by five experts, no other association between exposure and the ANCA biomarker was observed, even when solvents were considered as a group. The fact that self-reported dust exposure was significantly associated with ANCA might be explained through the high percentage of silica contained in dust.

Silica is widely used in many industries, including mining and quarrying (metallic and nonmetallic minerals), stone cutting, construction activities (tunnels and buildings) and sandblasting [34]. It is of note that seven out of 13 patients exposed to silica associated with ANCA-related disease in our study worked as bricklayers. In a previous study of occupational exposure to silica in individuals suffering from Wegener glomerulomatosis, three out of four patients exposed to silica were bricklayers [22]. This might thus be an occupational category at high risk of ANCA-related disease.

To evaluate the dose of silica on the incidence of ANCA we performed a quantitative analysis of the exposure. We observed a positive dose-effect relationship between silica exposure and the presence of ANCA, particularly concerning the intensity of exposure. This is in accordance with a previous study where a high silica exposure index was associated with an increased risk of primary vasculitis [30]. Accelerated apoptosis of macrophages or polynuclear neutrophils might thus be a dose-dependent effect.

The reasons why some individuals develop ANCA after silica exposure, and others do not, remain to be resolved. Genetic and/or infectious factors could play a crucial role.

In the present case-control study we evaluated the risk of occupational exposure in patients with a measurable marker, and not with a clinical entity which may be difficult to define. ANCA-positive patients and controls were recruited in the same hospital, thus limiting the risk of bias through control selection and nearly all patients and controls lived within a distance of 200 km from this hospital. The exclusion of 37 patients because of death or loss to follow-up may have introduced a bias. However, it is more likely that the drop-out of these patients would underestimate than overestimate the OR of the role of occupational exposure in this study.

CONCLUSION

Our study confirmed that environmental factors may be associated with the occurrence of autoimmune diseases. Occupational exposure, in particular to silica, might thus play a key role in the pathogenesis of occurrence and/or relapse of ANCA-related diseases and should be systematically explored in patients with such diseases.

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REFERENCES

1. Jennette JC, Falk RJ, Andrassy K, *et al*: Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 37:187–192, 1994
2. Wiik A: Granulocyte-specific antinuclear antibodies. Possible significance for the pathogenesis, clinical features and diagnosis of rheumatoid arthritis. *Allergy* 35:263–189, 1980
3. Halbwachs-Mecarelli L, Nusbaum P, Noel LH, *et al*: Antineutrophil cytoplasmic antibodies (ANCA) directed against cathepsin G in ulcerative colitis, Crohn's disease and primary sclerosing cholangitis. *Clin Exp Immunol* 90:79–84, 1992
4. Font J, Ramos-Casals M, Cervera R, *et al*: Antineutrophil cytoplasmic antibodies in primary Sjogren's syndrome: Prevalence and clinical significance. *Br J Rheumatol* 37:1287–1291, 1998
5. Zauli D, Ghetti S, Grassi A, *et al*: Anti-neutrophil cytoplasmic antibodies in type 1 and 2 autoimmune hepatitis. *Hepatology* 25:1105–1107, 1997
6. Parks CG, Cooper GS, Nylander-French LA, *et al*: Occupational exposure to crystalline silica and risk of systemic lupus erythematosus: A population-based, case-control study in the southeastern United States. *Arthritis Rheum* 46:1840–1850, 2002
7. Morita S, Ueda Y, Eguchi K: Anti-thyroid drug-induced ANCA-associated vasculitis: A case report and review of the literature. *Endocr J* 47:467–470, 2000
8. Savige J, Gillis D, Benson E, *et al*: International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am J Clin Pathol* 111:507–513, 1999
9. Russell KA, Wiegert E, Schroeder DR, *et al*: Detection of antineutrophil cytoplasmic antibodies under actual clinical testing conditions. *Clin Immunol* 103:196–203, 2002
10. Filer AD, Gardner-Medwin JM, Thambyrajah J, *et al*: Diffuse endothelial dysfunction is common to ANCA associated systemic vasculitis and polyarteritis nodosa. *Ann Rheum Dis* 62:162–167, 2003
11. Preston GA, Yang JJ, Xiao H, Falk RJ: Understanding the pathogenesis of ANCA: Where are we today? *Cleve Clin J Med* 69 (Suppl 2): S51–S54, 2002
12. Heeringa P, Brouwer E, Cohen Tervaert JW, *et al*: Animal models of anti-neutrophil cytoplasmic antibody associated vasculitis. *Kidney Int* 53:253–263, 1998
13. Esnault VL, Testa A, Audrain M, *et al*: Alpha 1-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. *Kidney Int* 43:1329–1332, 1993
14. Stegeman CA, Tervaert JW, de Jong PE, Kallenberg CG: Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med* 335:16–20, 1996
15. Duna GF, Cotch MF, Galperin C, *et al*: Wegener's granulomatosis: Role of environmental exposures. *Clin Exp Rheumatol* 16:669–674, 1998
16. Hogan SL, Satterly KK, Dooley MA, *et al*: Silica exposure in antineutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. *J Am Soc Nephrol* 12:134–142, 2001
17. Diot E, Lesire V, Guilmot JL, *et al*: Systemic sclerosis and occupational risk factors: A case-control study. *Occup Environ Med* 59:545–549, 2002
18. Lundberg I, Alfredsson L, Plato N, *et al*: Occupation, occupational exposure to chemicals and rheumatological disease. A register based cohort study. *Scand J Rheumatol* 23:305–310, 1994
19. Sanchez-Roman J, Wichmann I, Salaberri J, *et al*: Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. *Ann Rheum Dis* 52:534–538, 1993
20. Rasmussen N, Wiik A: Indirect immunofluorescence examination for IgG-ANCA in sera submitted for the 1st International Workshop on ANCA, 1988. *APMIS* 6:16–20, 1989
21. Leavitt RY, Fauci AS, Bloch DA, *et al*: The American College of Rheumatology, 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 33:1101–1107, 1990
22. Nuyts GD, Van Vlem E, Thys J, *et al*: New occupational risk factors for chronic renal failure. *Lancet* 346:7–11, 1995
23. Armitage P: Test for linear trend in proportions and frequencies. *Biometrics* 11:375–386,1955
24. Reinhold-Keller E, Herlyn K, Wagner-Bastmeyer R, *et al*: No difference in the incidences of vasculitides between north and south Germany: First results of the German vasculitis register. *Rheumatology (Oxford)* 41:540–549, 2002
25. Savage CO, Harper L, Cockwell P, *et al*: ABC of arterial and vascular disease: Vasculitis. *Br Med J* 320:1325–1358, 2000
26. Hogue CJ, Gaylor DW, Schulz KF: Estimators of relative risk for case-control studies. *Am J Epidemiol* 118:396–407, 1983
27. Gregorini G, Ferioli A, Donato F, *et al*: Association between silica exposure and necrotizing crescentic glomerulonephritis with p-ANCA and anti-MPO antibodies: A hospital-based case-control study. *Adv Exp Med Biol* 336:435–440, 1993
28. Nuyts GD, Van Vlem E, De Vos A, *et al*: Wegener granulomatosis is associated to exposure to silicon compounds: A case-control study. *Nephrol Dial Transplant* 10:1162–1165, 1995
29. Van Der Woude FJ: Taking anti-neutrophil cytoplasmic antibody (ANCA) testing beyond the limits. *Nephrol Dial Transplant* 17:2081–2083, 2002
30. Lane SE, Watts RA, Bentham G, *et al*: Are environmental factors importants in primary systemic vasculitis? A case-control study. *Arthritis Rheum* 48:814–823, 2003
31. Esnault VL: Apoptosis: The central actor in the three hits that trigger anti-neutrophil cytoplasmic antibody-related systemic vasculitis. *Nephrol Dial Transplant* 17:1725–1728, 2002
32. Leigh J, Wang H, Bonin A, *et al*: Silica-induced apoptosis in alveolar and granulomatous cells in vivo. *Environ Health Perspect* 105 (Suppl 5):S1241–S1245, 1997
33. Aikoh T, Tomokuni A, Matsukii T, *et al*: Activation-induced cell death in human peripheral blood lymphocytes after stimulation with silicate in vitro. *Int J Oncol* 12:1355–1359, 1998
34. Koeger AC: Exposure to silica causing connectivitis. *Presse Med* 23:11–14, 1994